Experimental cytomegalovirus infections in a rat model : pathogenesis and treatment

~ Chapter 8~

Summary and general discussion

Introduction

The pathophysiological and clinical characteristics of CMV infection are reviewed in **chapter 1**. Seroprevalence of CMV infections in the normal population is high and CMV infection usually follows a subclinical course. In contrast, patients with a defective immune system are at risk for developing severe CMV disease. For example, AIDS patients with a progressive decline in CD4+ T cell counts, suffer from severe CMV infections at CD4+ T cell counts below 100 cells/μl blood. Additionally, when immune effector functions are restored by HAART, the incidence of opportunistic infection, such as CMV, drastically diminishes.

CMV is able to infect a wide range of tissues and one of the main characteristics of CMV infection is the ability to persist at these sites, i.e. during chronic infection or latency. Peripheral blood mononuclear cells and their progenitors have been suggested as the main site of viral latency in humans, although CMV DNA can be found in multiple cell types in several organs of healthy trauma victims. Allogeneic stimulation of latently infected peripheral blood mononuclear cells has been shown to result in reactivation of virus. In addition, CMV seropositive patients receiving hemapoietic stem cell transplantation are at the highest risk for developing CMV disease, regardless of the CMV serostatus of the donor. These findings stress the importance of immune activation on RCMV replication and reactivation. Data from the murine model suggest that reactivation of virus from the lungs occurs stochastically via a patchwork pattern and does not depend on a decrease in immune effector functions. The significance of these findings for the human situation remains to be determined.

The interaction between CMV and the host's immune system is complex. It is generally accepted that atherosclerosis is an chronic inflammatory disease and develops as a response to injury. There is increasing evidence that systemic CMV infection or CMV replication in the atherosclerotic vascular wall itself accelerates atherosclerotic changes. CMV-mediated endothelial dysfunction and virus-mediated proliferation and migration result in increased inflammation, lipid accumulation and lumen narrowing. Additionally, CMV is thought to be an important risk factor for chronic rejection. The development of chronic rejection in the CMV-infected host is mediated by an increased and prolonged immune response, which is triggered by the viral challenge. On the other hand, however, TNF-α has been shown to directly enhance CMV replication via stimulation of the CMV IE enhancer/promotor mediated by transcription factor NF-κB. These data suggest that CMV replication enhances inflammation and vice versa.

Current treatment of CMV infections with either ganciclovir, foscarnet or cidofovir is effective, although drug resistant strains have been reported. Furthermore, evidence is increasing that also CMV-triggered inflammation may partly account for the observed tissue damage. This points out the need for new antiviral therapies in which antiviral

activity and the ability to modulate CMV-triggered immune activation are combined. Insight in viral pathogenesis is essential for developing new modalities of intervention. RCMV has been isolated approximately 20 years ago [78] and experimental RCMV infections have been accepted as models for CMV infections in humans.

RCMV replication in salivary gland cells

The salivary gland is the preferred organ for CMV infections. RCMV replication in salivary gland cells is detectable approximately 8 days after infection, whereas during the first week after infection, infectious virus is predominantly produced in internal organs, such as liver and spleen [75, 77, 497]. Infection of salivary glands is characterized by a long term production of high amounts of infectious virus. The salivary gland consists of three major glands, designated the submandibular, parotid and sublingual gland. Although some data were available about RCMV replication in the submandibular gland, little was known about virus replication in the parotid and the sublingual salivary gland. Replication of RCMV in the major salivary glands is described in more detail in ***chapter 2***. Assessment of viral load in the submandibular, parotid and sublingual gland of young adult animals, revealed that the submandibular gland was the preferred organ for RCMV replication. In contrast, in newborn rats, the main site of viral replication was the sublingual gland. It is possible that the maturation process of the sublingual gland enables optimal RCMV replication shortly after birth, whereas this permissiveness for the virus is lost when the maturation process is completed. Interestingly, it was shown by immunohistochemistry, *in situ* hybridization and electron microscopy, that, in all infected animals, viral replication was restricted to striated duct cells. This is in sharp contrast to MCMV where replication is exclusively found in acinar cells. Differences in cell tropism between RCMV and MCMV are especially fascinating since it is known that both viruses possess a lot of similarities and the genome of RCMV and MCMV show the highest homology of all known CMVs. Infection of rats resulted in a cellular inflammatory response, which was predominantly located in the interlobular duct region. Only a few inflammatory cells were found in the neighborhood of infected striated duct cells. A similar observation was made in the murine system [216]. The inability of inflammatory cells to reach infected striated duct cells may be one of the explanations for the persistence of CMV in the salivary glands.

It was shown that infectious virus was produced in large cytoplasmic vesicles and is excreted into the lumen of the salivary gland by apical cell budding of the vesicle. The chronic production of high amounts of infectious virus could have other consequences, rather than solely providing infectious virus for horizontal transmission. Shedding of virus from the salivary gland may result in re-infection of the host. In order to study the biological significance of RCMV replication in the salivary gland cells, we employed a model in which the salivary glands were removed in a subset of rats prior to infection ***(chapter 3)***.

Surgically desalivated rats were compared to sham-operated animals after a lethal and sublethal infection with RCMV, respectively. Survival was recorded and presence of infectious virus was determined by plaque assay and the presence of viral DNA was assessed by PCR. We demonstrated that desalivation did not have an effect on the clinical course of RCMV disease and on RCMV-induced mortality, but a significantly higher titer of anti-RCMV antibodies was found in sham-operated rats than in desalivated animals during long-term follow-up. These data indicate that shedding of virus or viral antigens to the systemic circulation occurs from the salivary gland via virus-containing saliva and that this re-introduction of virus or its components is responsible for boosting the humoral response, resulting in high antibody titers. Interestingly, we were unable to detect viral DNA and infectious virus in spleen, liver and lung of infected animals at 1 year p.i., but as expected, the submandibular glands still yielded high amounts of infectious virus. These data suggest that boosting of the humoral response is caused by shedding of viral antigens from the salivary glands and that infectious virus shed from the salivary gland is neutralized by already existing circulating anti-RCMV antibodies, a phenomenon described previously [250].

The r144 gene product interferes with the innate immune system

CMV infection is the most common congenital infection in humans. Approximately 5% of infected newborns are born with CMV disease [128, 393]. Prognosis in this group is poor and the clinical presentation during follow-up is mainly characterized by neurological deficits [106, 387]. A significant proportion of the asymptomatic infected newborns (5%) will suffer from long term sequelae of which sensorineural hearing loss is most common [166, 167]. Sensorineural deterioration due to asymptomatic infection usually progresses undetected and it has been reported that screening of these patients fails in over 50% of the cases [166]. In contrast to the human situation, RCMV is not able to cross the placental barrier (unpublished results). It is therefore likely that vertical transmission of virus in rats is of little importance and that newborn rats become infected after birth. Thus, an experimental rat model for congenital infection in humans can only be achieved by infecting newborn rats shortly after birth. Experimental RCMV infection in newborn rats is described in **chapter 4**. RCMV infection of newborn rats resulted in similar distribution of virus during the course of infection as did infection of immunocompromised adult rats. RCMV was present in liver, spleen and lung at 3 and 5 days p.i., whereas RCMV could only be detected in the salivary glands at 21 days p.i. Furthermore, RCMV infection resulted in an inflammatory response in infected organs, which consisted of monocytes/macrophages, T cells and NK cells. The immune system of neonatal rats was able to control CMV infection to some extent, since animals did not show symptoms of CMV disease and did survive for at least 21 days after inoculation of 10^4 PFU of virus. In

contrast, it is known that survival rates are greatly impaired when rats of 4 weeks old are subjected to 5 Gy of TBI prior to injection of 10^5 PFU of RCMV. The usage of TBI as immune suppressive regimen affects the number of granulocytes, NK cells, monocytes, B cells and T (CD4+ and CD8+) cells in peripheral blood of irradiated animals significantly and leads to a transient state of severe immunosuppression [525]. Peripheral white blood cells are suppressed during the acute phase of infection and the number of cells return to control levels at approximately 3-4 weeks after irradiation [525]. The natural, unaffected immune system of neonatal rats enables disseminated RCMV infection, but prevents uncontrolled viral replication and CMV-induced multi-organ failure. This optimal equilibrium between on the one hand the level of RCMV replication and on the other hand the control of RCMV infection by immune effector functions in neonatal rats enables us to study the interaction of the virus with the immune system of the host.

CMV has developed several mechanisms to evade immune surveillance [353]. One of those mechanisms is to downregulate cellular MHC class I expression. However, according to the 'missing self hypothesis', downregulation of MHC class I renders a cell susceptible to NK cell killing [316]. Viral encoded MHC class I homologs have been identified on the genome of human CMV (UL18, [39]), MCMV (m144, [155]) and RCMV (r144, [43]).

An RCMV strain in which the r144 gene is disrupted was generated in our laboratory [43] and infection of immunocompromised adult rats with this deletion mutant showed that there were no significant differences in tissue distribution and virus titers between wt RCMV- and RCMVΔr144-infected animals [43]. Since it is thought that the r144 gene might play a role in virus-induced evasion of immune surveillance, it is possible that the high level of irradiation-induced immunosuppression was responsible for those results. In order to study the role of r144 in the pathogenesis of RCMV infection in rats with a normal unaffected immune system, neonatal rats were challenged with either wt RCMV or RCMVΔr144 and the presence of infectious virus as well as viral DNA was assessed in various organs at different time points p.i. Replication of RCMVΔr144 in the spleen and the salivary gland was severely restricted compared to wt RCMV at 3 and 21 days p.i., respectively. RCMVΔr144, has previously been shown to have similar replication characteristics as wt virus *in vitro* as well as *in vivo* [43], suggesting that RCMVΔr144 replicates as efficiently as wt RCMV in the absence of a fully functional immune system. In addition, also a significant lower number of monocytes/macrophages and NK cells were found in spleens of RCMVΔr144-infected rats than in those of wt RCMV-infected animals at 3 days p.i. These results are in concert with previous results, using a local infection model, in which either RCMVΔr144 or wt RCMV were subcutaneously injected in the hind paw of immunocompromised adult rats. In this model, both viruses were shown to replicate with similar efficiency locally in the hind paw. However, a significantly lower number of

monocytes/ macrophages was found at the site virus injection. In this case, however, a similar number of NK cells was found in the rat hind paw of RCMVΔr144- than in those of wt RCMV-infected animals. These data from the neonatal- and rat hind paw model are in concert with previous reports obtained from the human [116, 306] and murine system [282]. The UL18 gene product was shown to interact with a membrane receptor, designated LIR-1, which is predominantly expressed on monocytes and B lymphocytes, but only on a minor subset of NK cells [114]. Cretney et al. showed that the m144 gene product provided some protection against NK cell-mediated killing of lymphoma cells transfected with m144 *in vitro*, but that *in vivo*, the major effect of m144 is to regulate NK cell accumulation and activation [116]. These data show that the CMV-encoded MHC class I homolog is more likely to compromise the innate immune system as a whole, rather than to solely inhibit NK cell-mediated killing.

RCMV infection of renal allografts results in an increased and prolonged expression of adhesion molecules.

Current immunosuppressive regimens and anti-rejection therapies prevent renal allograft failure due to acute rejection in the majority of cases. However, several years after transplantation, a significant number of renal allografts are lost as a result of chronic rejection. The level of histoincompatibility, inadequate immunesuppression, acute rejection and viral infections, especially CMV infections, have been suggested as risk factors for chronic rejection [14, 38, 209, 390]. Although it is generally accepted that CMV infection plays a role in the acceleration of chronic rejection [20, 193, 277, 281, 292, 339, 380] the mechanism involved is unclear. To study the role of CMV infection chronic renal allograft rejection, the rat model developed by Soots et al. [483] was used.

Chapter 5 focuses on the accelerated development of chronic rejection in RCMV infected rats receiving a kidney allograft under triple drug immunosuppression. It was shown previously that renal allografts harvested from rats receiving triple drug immunosuppression showed histological signs of chronic rejection at 40-60 days after transplantation [483]. The histological diagnosis of chronic rejection includes perivascular and interstitial inflammation, fibrosis, glomerulosclerosis, vascular intimal thickening and tubular atrophy [391, 480]. The peak of inflammation in these allografts was seen 5-10 days p.i. and was associated with lymphoid activation and induction of adhesion molecules [259]. Thereafter, renal inflammation gradually decreases while the histological characteristics of chronic rejection became more and more prominent. RCMV antigens could be detected in endothelial cells of renal allografts at 7 days p.i. [294]. Strikingly, RCMV infection increased and prolonged inflammation and accelerated the development of chronic rejection significantly. The histological criteria for chronic rejection were already fulfilled before 20 days after transplantation [294]. RCMV infection of renal allografts was associated with an

increased and prolonged expression of adhesion molecules ICAM-1 and VCAM-1 on endothelial cells and their ligand molecules, LFA-1 and VLA-4, expressed on leukocytes. Expression of adhesion molecules is mediated via cytokines, such as IL-1, TNF-alpha and IFN-gamma, which are produced by activated lymphocytes. On the other hand, CMV has been shown to upregulate IL-1β gene expression, which may lead to production of IL-1 by mononuclear cells [235]. In addition, IE genes of CMV have been found to upregulate IL-2 and IL-2R genes [179]. Furthermore, CMV induces TNF-alpha in monocytes/macrophages [472], as well as *in vivo* [202]. RCMV mediated production of cytokines may result in a generalized increased and prolonged expression of adhesion molecules, even on uninfected endothelial cells of the graft [541]. These data suggest that RCMV influences the induction phase of the alloresponse and determines the magnitude of this process from that point onward.

Treatment of CMV infections in immunocompromised rats.

Despite the drastic decrease in CMV related morbidity since the introduction of HAART, CMV-induced encephalomeningitis and its treatment are still a major clinical problem in patients with end-stage HIV infection. The effect of several antiviral compounds on RCMV-induced encephalomeningitis in immunocompromised rats is described in ***chapter 6***. Rats were intracerebrally infected and were systemically treated with either HPMPC, DHPG, HIS, or DHPG in combination with HIS. After intra-cerebral infection, the viral antigens could be detected in mononuclear cells in the meninges and endothelial cells of small vessels. In addition, an inflammatory response consisting of mononuclear cells and T cells and scattered foci of necrosis harboring RCMV antigens could be demonstrated in the meninges and to a lesser extent in the brain. RCMV infection of endothelial cells is associated with a local inflammatory response, in a similar manner as described previously in the rat hind paw model [399]. The observed histological alterations in our rat model closely resemble the pathology of CMV-induced meningoencephalitis in AIDS patients [208, 360]. Viral replication in endothelial cells may be a portal for entry of CMV into the central nervous system. Treatment with a single dose of 20 mg/kg HPMPC significantly reduced the amount of infectious virus in brain and spleen compared to all other treatment modalities, which were administered daily at the appropriate doses for 5 days. One explanation for this therapeutic failure in the model used could be poor penetration of the antiviral compounds into the brain area, which permits initial replication cycles of the virus.

Tissue damage due to CMV infection is at one hand caused by direct virus-induced cell damage and on the other hand mediated by the mounted immune response. For example, the HAART-associated reconstruction of immune effector functions in AIDS patients renders patients with a history of CMV retinitis susceptible to IRV, a condition

characterised by retinal inflammation without any signs of CMV relapse [256, 257]. In addition, CMV pneumonitis in AIDS patients is rare and follows a mild course, whereas CMV pneumonitis in BMT is a frequent and lethal condition [37]. This suggest that the inflammatory immune response after CMV infection plays an important role in causing tissue dysfunction. Drugs with anti-CMV and anti-inflammatory properties may be beneficial. ***Chapter 7*** describes the evaluation of 2 compounds, DFO and DTPA, which are thought to inhibit viral replication and decrease oxidative stress. The mechanism by which both compounds act is not fully understood, but it is thought that the inhibition of an iron-dependent ribonucleotide reductase may be responsible for the inhibition of RCMV replication. The antiviral effect of both compounds was assessed *in vitro* as well as *in vivo*, using a generalized and a local infection model. In concert with previous results [103, 105], DFO and DTPA exhibited an antiviral effect *in vitro*, although DTPA was more potent than DFO. In immunocompromised rats, DFO and DTPA were unable to prevent RCMV mortality and RCMV disease. Neither DFO nor DTPA reduced virus replication in organs significantly after a sublethal RCMV challenge. A local infection model, in which RCMV was injected in the rat hind paw of immunocompromised animals, enabled us to study the effect of DFO and DTPA on the spread of virus from the infection site as well as the effect of both drugs on the local inflammatory response. Treatment with DFO or DTPA was unable to reduce the swelling of the hind paw, did not affect the viral load as measured by the number of RCMV antigens expressed in rat hind paw tissue and failed to diminish the number of infiltrating leukocytes. However an antiviral effect was observed in the local infection model, as shown by a significant decrease in the number of rats harboring infectious virus in spleen and liver after treatment with DFO or DTPA. The in vivo transplantation study using rat liver allografts by Martelius and co-workers [331] supported this finding. In their study an inhibitoy effect of metal chelators on CMV replication, inflammation an on bile-duct damage was detected. In addition, DFO has been shown to reduce auto-immune-mediated retinal inflammation [252] and iatrogeneic inflammation of the rat hind paw [53] significantly. Both compounds were shown to inhibit 1) mitogen- and allogen- induced proliferation of peripheral blood lymphocytes, 2) NK cell function and 3) adhesion molecule expression [450]. More importantly, in a case report, administration of DFO to an AIDS patient suffering from CMV retinitis, refractory to ganciclovir and foscarnet, inhibited progression of ocular changes. These data indicate that metal chelators as DFO and DTPA have to some extent antiviral capacities *in vivo*, but that these compounds as reported by Martelius et al. are especially usefull in settings with evident immune activation, such as organ transplantation recipients.

Concluding remarks

In this thesis several aspects of CMV infection and CMV disease have been studied in a rat model. The model used in this thesis gave us the opportunity to study the replication of the virus and the pathology it induced in more details. Although, RCMV infections closely resemble CMV infections in man, one should bear in mind that translation of data from CMV-infected laboratory animals to the human situation should be made with care, even when the genome of both strains shows a high level of homology. Nevertheless, the ability to control most variables in an experimental setting using animals and the appropriate viruses enable us to unravel mechanisms of CMV disease. Data from these models may provide new potential targets for antiviral treatment.

Samenvatting

De seroprevalentie van CMV is hoog in de gezonde populatie. Een eerste infectie met CMV is normaliter asymptomatisch. In een minderheid van de gevallen kan een griepachtig beeld ontstaan. Levensbedreigende ziekte door CMV infectie bij gezonde personen komt voor, maar is uiterst zeldzaam. Patiënten met een immuundeficiëntie, zoals AIDS- of transplantatiepatiënten, lopen risico op ernstige CMV ziekte door een primaire infectie of reactivatie van latent aanwezig CMV. Ganciclovir, cidofovir en foscarnet zijn middelen die gebruikt worden voor de behandeling van ziekte door CMV infectie.

Ongeveer 20 jaar geleden werd uit ratten CMV geïsoleerd (RCMV). Het RCMV model wordt gebruikt voor onderzoek naar pathogenese en het testen van nieuwe potentieel antivirale middelen. In dit model wordt het virus meestal intraperitoneaal toegediend, maar ook subcutane en intraveneuze toediening kunnen gebruikt worden. Na intraperitoneale toediening wordt het virus in de eerste week na infectie voornamelijk gevonden in abdominale en thoracale organen zoals lever, milt, nieren en longen. Na de eerste week kan geen infectieus virus in deze organen meer worden aangetoond. Wel blijft het DNA van CMV in deze organen aantoonbaar (latentie). Vanaf ongeveer 8 dagen na infectie wordt er infectieus virus aangetroffen in de speekselklieren. De productie van infectieus virus houdt lange tijd (enkele maanden) aan. ***Hoofdstuk 2*** beschrijft de replicatie van RCMV in de speekselklieren van de rat. Met in situ hybridisatie, electronen microscopie en immunohistochemie is gebleken dat, ondanks verschillen in speekselkliertropisme tussen pasgeboren- en jongvolwassen ratten, uitsluitend striatale cellen geïnfecteerd zijn. Het virus wordt met het speeksel uitgescheiden. Dit is een van de mechanismen waarmee het virus zich kan verspreiden tussen soortgenoten. Het is onduidelijk of het uitscheiden van speeksel met CMV een re-infectie veroorzaakt. In ***hoodstuk 3*** worden ratten met en zonder speekselklieren geïnfecteerd met RCMV om het infectieverloop in beide groepen te vergelijken. Het acute infectieverloop na een letale dosis RCMV verschilt niet in ratten met en zonder speekselklieren. In een tweede experiment, waarbij ratten gedurende een jaar gevolgd werden, werd een verschil in antilichaamtiter gevonden. Ratten zonder speekselklieren hadden lagere antilichaamtiters na infectie dan ratten met speekselklieren. Dit suggereert dat uitscheiding van het virus of virale antigenen plaatsvindt via speekselklieren en dat dit mede verantwoordelijk is voor activatie van de humorale immuunrespons.

In tegenstelling tot jongvolwassen ratten heeft men bij pasgeboren ratten geen algehele lichaamsbestraling nodig om een ernstig verlopende RCMV infectie te bewerkstelligen. Hoewel het immuunsysteem van pasgeboren ratten nog onrijp is, is het echter in staat om de infectie uiteindelijk het hoofd te bieden en overlijden te voorkomen. Het gebruik van pasgeboren ratten is dus een uitermate geschikt model voor het bestuderen van de interactie van het virus met het immuunsysteem. Eerder onderzoek

wijst erop dat een aantal genen in het CMV genoom coderen voor eiwitten die interfereren met het immuunsysteem. Door het immuunsysteem te misleiden en een geïnfecteerde cel als "gezond" te presenteren kan de virusgeïnfecteerde cel ontsnappen aan het immuunsysteem en er derhalve voor zorgen dat het virus overleeft. De door CMV gecodeerde MHC klasse I homoloog is geassocieerd met het proces van immuun evasie. Het cellulaire MHC klasse I eiwit wordt tot expressie gebracht op de celmembraan van elke kernhoudende lichaamscel en speelt een cruciale rol in de afweer tegen virusinfecties. In ***hoofdstuk 4*** wordt de bijdrage van de virale gecodeerde MHC klasse I homoloog aan de pathogenese nader bestudeerd door virussen met (wt RCMV) en zonder de MHC klasse I homoloog (RCMVΔr144) met elkaar te vergelijken. Hiervoor werden pasgeboren ratten intraperitoneaal geïnfecteerd met een van beide virussen. Eerder onderzoek liet zien dat beide virussen in vitro even efficiënt repliceerden. Daarnaast konden er geen verschillen in virusconcentratie worden aangetoond tussen wt RCMV- en RCMVΔr144-geïnfecteerde immuundeficiënte ratten. In deze experimenten werd de virale replicatie niet beïnvloed door een aanwezig immuunsysteem. Echter in het RCMV-infectiemodel met pasgeboren ratten wordt er een significant lagere hoeveelheid infectieus virus gevonden in de milt en speekselklieren van RCMVΔr144- dan in milt en speekselklieren van wt RCMV-geïnfecteerde ratten. Deze resultaten in de verschillende modellen suggereren dat het verschil in virusreplicatie tussen beide virussen veroorzaakt wordt door de activiteit van het immuunsysteem. De functie van de virale MHC klasse I homoloog is echter nog onduidelijk. Er zijn aanwijzingen dat de viraal gecodeerde MHC klasse I homoloog, naast zijn functie als vervanger van het cellulaire MHC klasse I, ook een interactie aangaat met cellen van het niet-specifieke immuunsysteem.

CMV speelt een belangrijke rol bij orgaantransplantaties. De mate van histo-incompatibiliteit, inadequate immunosuppressie, episodes van acute afstoting en virale infecties (waaronder CMV) worden geassocieerd met de chronische afstotingsreactie. Hoewel het verband tussen CMV infectie en chronische afstoting algemeen geaccepteerd is, is het achterliggende mechanisme onduidelijk. Uit eerder onderzoek in het RCMV model is gebleken dat RCMV infectie de chronische afstotingsreactie van een allogeen niertransplantaat versnelt. De versnelde afstotingsreactie door RCMV gaat gepaard met verhoogde expressie van de adhesiemoleculen ICAM-1 en VCAM-1 op endotheelcellen van de getransplanteerde nier en hun ligand moleulen LFA-1 en VLA-4 op de aanwezige leukocyten (***Hoofdstuk 5***). Het is bekend dat CMV de expressie van sommige cytokines, zoals IL-1β, IL-2, IL-2R en TNF-α, kan verhogen. De langdurig verhoogde expressie van adhesiemoleculen op geïnfecteerde en niet-geïnfecteerde endotheelcellen kan verklaard worden door de verhoogde cytokine productie. Adhesiemolecuul expressie is essentieel voor het uittreden van inflammatoire cellen uit de bloedbaan en staat als dusdanig aan de basis van de afstotingsreactie van de getransplanteerde nier.

De behandeling van ziekte door CMV infectie is complex. Aangezien CMV infectie voornamelijk een probleem is in immuundeficiënte patiënten zou theoretisch de beste behandeling het opheffen van de immuundeficiëntie zijn. Een voorbeeld hiervan betreft het behandelen van AIDS patiënten met HAART. Door de replicatie van HIV te reduceren zal het immuunsysteem (T-helper cellen) van de patiënt tijdelijk kunnen herstellen. De incidentie en de ernst van CMV infecties in deze populatie daalt dan drastisch. In een aantal patiënten wordt echter een immunologisch gemedieerde orgaanschade gemeld. Dit treedt voornamelijk op ter hoogte van de retina. Het immuunsysteem gaat dan massaal het geïnfecteerde weefsel te lijf en schiet hierbij zijn doel voorbij waardoor zogenaamde immuunpathologische schade optreedt. In ***hoofdstuk 6*** wordt de werking van enkele antivirale middelen bestudeerd. Deze middelen remmen direct de virale replicatie. Om dit te onderzoeken werden immuundeficiënte ratten intracerebraal geïnfecteerd met RCMV en vervolgens behandeld met HPMPC (cidofovir), DHPG (ganciclovir), Hyper Immuun Serum (HIS) en een combinatie van HIS en DHPG. In het beschreven ratmodel kwam het histopathologisch beeld van de hersenen overeen met de pathologie zoals die gezien wordt in CMV-geïnduceerde meningo-encephalitis in AIDS patiënten. Behandeling met HPMPC was, met betrekking tot inhibitie van virale replicatie, superieur aan de andere geteste antivirale middelen. Daarbij werd HPMPC slechts eenmalig gegeven, terwijl DHPG gedurende 5 dagen dagelijks toegediend werd. In ***hoofdstuk 7*** worden de resultaten beschreven van experimenten waarbij middelen getest worden waarvan naast een directe antivirale werking, ook een immuunmodulerende werking worden toegeschreven. DFO en DTPA zijn metaalchelatoren en hun antivirale werking wordt toegeschreven aan inhibitie van een ijzerafhankelijk ribonucleotide reductase. Gebruik van deze middelen in RCMV-geïnfecteerde ratten toont aan dat het antivirale effect van beide middelen in ratten gering is: DFO en DTPA konden overlijden ten gevolge van een letale dosis CMV niet voorkomen. Ook waren beide middelen niet in staat om de concentratie van infectieus virus in organen van niet-letaal geïnfecteerde ratten te verminderen. Wel werd er een beperkt antiviraal effect gezien van beide middelen in een lokaal infectiemodel. In dit model wordt virus subcutaan in de achterpoot van een immuundeficiënte rat gespoten. Infectieus virus wordt nu geleidelijk aan de lichaamscirculatie afgegeven. Behandeling met DFO en DTPA resulteerde in een significante vermindering van het aantal dieren met geïnfecteerde interne organen zoals lever en milt in vergelijking met controle dieren die geen behandeling kregen. DFO en DTPA hebben dus een beperkte waarde als antiviraal middel. Deze resultaten staan echter in schril contrast met de resultaten die door de groep van Lautenschlager verkregen werden in een allogeen levertransplantatiemodel. DFO bleek in dit model de afstotingsreactie en de virale replicatie te verminderen. Dit suggereert dat metaalchelatoren geïndiceerd kunnen zijn in patiënten met een duidelijke immuunactivatie. Verder onderzoek naar de werking van metaalchelatoren in

transplantatiemodellen is geïndiceerd.

In dit proefschrift worden enkele aspecten van CMV infectie en CMV ziekte bestudeerd in een ratmodel. De ratmodellen die in dit proefschrift gebruikt werden, gaven ons de mogelijkheid om de virale replicatie en de pathologie die het induceert te bestuderen. Hoewel ziekte door RCMV en CMV niet veel van elkaar verschillen, is echter voorzichtigheid geboden bij het extrapoleren van gegevens uit het RCMV model naar de humane situatie. Het voordeel van diermodellen is de mogelijkheid om veel variabelen te controleren en systematisch de mechanismen van CMV ziekte te ontrafelen.